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Interaction of the earthworm *Diplocardia mississippiensis* (Megascolecidae) with microbial and nutrient dynamics in a subtropical Spodosol

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Abstract

Direct and indirect effects of earthworm feeding and activity can alter soil microbial and nutrient dynamics. Little is known about influence of native North American earthworms on these dynamics. We investigated effects of native *D. mississippiensis* activity on total and soluble C, N and microbial biomass pools in Spodosols from the Apalachicola National Forest in North Florida, USA. Soil native to *D. mississippiensis* was collected and reconstructed by horizon (A, E, B_h) in laboratory chambers and incubated with earthworms at zero, low or high density, respectively 0, three, or six individuals, for 28–31 days. Total C and N were measured by dry combustion methods. Soluble C and N and microbial biomass were measured in 0.5 K_2SO_4 extracts from non-fumigated and fumigated soil samples. Substrate induced respiration (SIR) was used with inhibitors to measure microbial respiration and shifts in bacterial and fungal components. Earthworm activity mixed soil horizons, largely through burrowing and casting. Total C in A and B_h horizons averaged 16.7 mg C g^{-1} soil and was significantly greater than in the E (2.6 mg C g^{-1} soil). Soil N concentration was highest in the A (0.73 mg g^{-1} soil) and lowest in the E (0.11 mg N g^{-1} soil). N concentration was significantly increased over two fold in the E with high earthworm density. Microbial biomass C was greatest in the A and E, averaging 8.6 mg g^{-1} C, and decreased with earthworm activity. In all horizons, soluble C and N also increased with earthworm density. Net N mineralization increased significantly with earthworm density and ranged from –0.22 to 68 mg g^{-1} N across all treatments and layers. Changes in proportion of soluble to total soil nutrients indicated that greater turnover and mineralization occurred with earthworm activity. SIR indicated earthworm activity induced microbial respiration in A and E horizons. Earthworm activity stimulated fungal and bacterial respiration, respectively, by 13 and 30% in the A and by 123% and 450% in the E. Although bacterial respiration was stimulated more than fungal there was no significant shift in B:F ratio, which ranged from 0.80 to 0.99. SIR did not provide evidence of any selective microbial effects of earthworm activity. Direct and indirect effects on nutrient dynamics by earthworms were induced by casting activity and were associated with passage of material through the gut (direct effect) and nutrient and microbial enrichment through the mixing of soil horizons (indirect effect). This study established the importance of *D. mississippiensis* to nutrient availability in its native soil.

Keywords: Native earthworm; Nearctic earthworm; Nutrient dynamics; Microbial biomass; Substrate induced respiration; Apalachicola National Forest

1. Introduction

Earthworm activity, namely comminution, burrowing and casting in the soil, indirectly affects microorganisms (Brown, 1995). Earthworm activity modifies soil structure, moisture and aeration, redistributes carbon resources, and aggregates soil with castings (Brown, 1995; Doube and Brown, 1998). Direct effects of earthworms on microorganisms are caused by feeding on and dispersing microbial propagules (Brown, 1995). Devliegher and Verstraete (1995) characterized indirect and direct effects with respect to nutrient-enrichment (NEP) and gut-associated (GAP) processes. GAP may encompass increased soluble organic matter, microbial activity, and colony forming units of microorganisms observed in ingested material during gut passage (Parle, 1963a,b; Barois and Lavelle, 1986; Barois, 1992; Kristufek et al., 1992). Fischer et al. (1995) proposed that dormant bacterial cells were activated during gut passage. Bacterial activity can continue in fresh casts (Parle, 1963a; Daniel and Anderson, 1992) and result in local increases of total microbial activity in bulk soil. Fungal colonization and succession of species occurs in aging casts (Parle, 1963a; Tiwari and Mishra, 1993).

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Earthworm feeding activity may influence microbial dynamics. Some experiments report selective consumption of fungi over bacteria (Cooke and Luxton, 1980; Brown, 1995; Doube and Brown, 1998). Selective assimilation or stimulation of fungi and bacteria can influence long term microbial community dynamics in bulk soil (Scheu and Parkinson, 1994; McLean and Parkinson, 2000).

Activity of native North American earthworms and their direct or indirect interactions with the microbial community are little studied. Previous field results suggested geophagic feeding behavior of native southeastern earthworms (Megascolecidae: *Diplocardia* Garman, 1888) based on $\delta^{13}C$ signatures which indicated soil organic matter assimilation by these earthworms (Hendrix et al., 1999). Although, soil organic matter encompasses microbial $\delta^{13}C$ signatures selective feeding on microbial tissues could not be resolved. The objective of the present study was to determine the impact of the activity of *Diplocardia mississippiensis* Smith, 1924 on microbial community dynamics by examining carbon and nitrogen turnover through soil organic matter and microbial pools. We hypothesized that *D. mississippiensis* would have greater selective effects on fungal than on bacterial dynamics. *D. mississippiensis* may ingest fungi, which release oxalates, to derive calcium, a potentially limiting nutrient in the Apalachicola National Forest (ANF). Capability to ingest and metabolize oxalates binding calcium (Khambata and Bhat, 1955; Cromack et al., 1977; Graustein et al., 1977) has been suggested in *Arctiostrotus* sp., another Nearctic megascolecid earthworm (Spiers et al., 1986). Observing a direct impact of earthworms on microbes is usually confined to short term laboratory feeding experiments, therefore an attempt was made to determine selective effects of activity on bacterial:fungal (B:F) ratios using substrate induced respiration (SIR) with inhibitors (Anderson and Domsch, 1973). SIR was successfully used to show changes in B:F ratios due to earthworms under laboratory conditions (Scheu and Parkinson, 1994). Our analyses were made through comparison of zero, low and high earthworm densities.

2. Materials and methods

2.1. Site description

The system under study is the Apalachicola National Forest (ANF) located in the panhandle region of northwest Florida, USA. The ANF system is dominated by longleaf pine (*Pinus palustris*) with a shrub–grassland understory dominated by wire grass (*Aristida stricta*). More information on the ecology of this habitat can be obtained from Vail (1972); Callaham and Hendrix (1998); Hendrix et al. (1999). The USDA forest service manages this area with a prescribed burning program for the risk of forest fires. Vail (1972) recorded presence of three native species of *Diplocardia* (Megascolecidae) in the ANF. Reynolds (1994); Hendrix et al. (1999) confirmed the distribution of *D. mississippiensis,* and *D. floridana* in the ANF area. *D. komareki* may also occur but is rarely found (M.A. Callaham Jr., personal communication). Hendrix et al. (1994) reported that population densities of *Diplocardia* spp. changed seasonally and were reduced by earthworm harvesting in burn areas for the fishing bait industry. Hendrix et al. (1994) recorded greatest fresh biomass of 6.0 ± 1.0 g m^{-2} attributed to a density of one–three earthworms m^{-2}. *Diplocardia* sp. in this system likely forage on soil microbial biomass and decayed organic material (Vail, 1972; Hendrix et al., 1994). The geophagous feeding behavior of both *D. mississippiensis* and *D. floridana* was suggested by stable isotopes (Hendrix et al., 1999) and gut content analysis.

Soils in the area of ANF used for this study are acidic ($pH = 4.3$) and classified as a sandy, siliceous, thermic Aeric Alaquod. Three soil horizons were distinguished for this study. The A horizon, from 0 to 17 cm depth, occurs as a dark gray fine sand. The E horizon at 17–29 cm depth, is a light gray fine sand with little organic materials. The third, a B_h horizon occurring at 29–70 cm depth is an organic matter-rich very dark brown fine sand. The depths of these horizons are variable along a topographical sequence. The soil nutrient and microbial status of each soil horizon collected from the field and after an initial week long conditioning of reconstructed soil horizons at increased moisture content are given in Tables 1 and 2.

2.2. Experimental design

Soils from the three horizons (A, E and B_h) were retrieved along with native *Diplocardia* sp. from the ANF in management Compartment 47 in June 1998. Sieved (1 cm mesh) soil from each horizon was reconstructed as a unit in 15 PVC cylinders (15 cm dia, 30 cm height). Soil was packed at field bulk density, 10 cm thick each for A and E horizons, 5 cm thick for B_h, and wetted with deionized water to increase moisture to 25% gravimetric weight. After 1 week of conditioning, three units were sampled for preliminary studies. Earthworms were then added into two-thirds of the remaining units to generate three final treatments with four replicates for each treatment. The treatments were as follows: zero density treatment (ZDT) with no earthworms; low density treatment (LDT) with three earthworms; and high density treatment (HDT) with six earthworms. Treatments were incubated at constant moisture and temperature (20 ± 1°C) in the laboratory. Three units, one replicate from each treatment, were deconstructed on each day over days 28–31 of incubation and analyzed for microbial biomass and substrate induced respiration.

Total and extractable soil C and N were determined on each layer in every incubation cylinder and are expressed per gram oven dry soil weight (105°C). Total soil C and N were determined by dry combustion on a Carlo Erba CN analyzer. Soluble C and N were extracted with 0.5 M K_2SO_4 at 3:1 solution to soil mass ratio. Soluble organic C (C_{sol})

Table 1
Total soil N, inorganic N (NH_4^+-N + NO_3^--N), microbial biomass N (MBN), and net mineralized N, of initial and incubated soils by layer (A, E, and B_h) and by earthworm density treatment (ZDT = zero, LDT = three earthworms, and HDT = six earthworms). (Data are given as means (s.e.); for Initial $n = 2$, for earthworm density treatments $n = 4$)

	Layer		
	A	E	B_h
Total N (mg g^{-1} soil)			
Initial	0.71 (0.02)	0.06 (0.00)	0.48 (0.00)
ZDT	0.74 (0.01) Aa[a]	0.07 (0.003) Ba	0.49 (0.01) Ca
LDT	0.71 (0.03) Aa	0.12 (0.003) Bb	0.50 (0.01) Ca
HDT	0.73 (0.03) Aa	0.14 (0.003) Bc	0.53 (0.02) Ca
Inorganic N (mg g^{-1} N)			
Initial	3.2 (0.2)	11.0 (0.3)	2.7 (0.1)
ZDT	4.4 (0.3) Aa	10.5 (0.5) Ba	2.5 (0.5) Ca
LDT	21.2 (2.9) Ab	34.4 (5.3) Bb	11.2 (3) Cb
HDT	33.4 (3.5) Ac	72.6 (20.5) Bc	42.7 (11.6) Cc
MBN (mg g^{-1} N)			
Initial	29.7 (14.6)	37.5 (17.6)	11.3 (2.0)
ZDT	21.7 (1) Aa	20.5 (3.9) Aa	10.2 (1) Ba
LDT	12.4 (2.6) Ab	10.7 (4) Ab	13.7 (1.4) Aa
HDT	18.2 (1.7) Aab	7.2 (5) Bb	14.0 (3.8) ABa
Net mineralized N (mg g^{-1} N)			
ZDT	1.35 (0.32) Aa	0.14 (0.44) Aa	−0.22 (0.42) Ba
LDT	17.99 (2.82) Ab	28.04 (5.26) Ab	9.13 (2.98) Bb
HDT	30.29 (3.88) Ac	67.63 (20.74) Ac	40.28 (23.28) Bc

[a] Significant differences within each pool, by multiple comparison (Fisher's LSD test, $P < 0.05$), among layers (within treatment row) are indicated by capital letters and among treatments (within layer column) are indicated by lower case letters.

Table 2
Total soil C, soluble organic C, and microbial biomass C (MBC), of initial and incubated soils by layer (A, E, B_h) and by earthworm density treatment (ZDT = zero, LDT = three earthworms, and HDT = six earthworms). (Data are given as means (s.e.); for Initial $n = 2$, for earthworm density treatments $n = 4$)

	Layer		
	A	E	B_h
Total C (mg g^{-1} soil)			
Initial	16.5 (0.5)	1.6 (0.1)	16.1 (0)
ZDT	17.5 (0.3) Aa[a]	1.71 (0.5) Ba	16.2 (0.5) Aa
LDT	16.6 (0.7) Aa	2.72 (0.14) Ba	16.5 (0.6) Aa
HDT	17.1 (0.7) Aa	3.35 (0.08) Ba	16.4 (0.6) Aa
Soluble organic C (mg g^{-1}C)			
Initial	1.8 (0.1)	9.1 (0.7)	4.1 (0.6)
ZDT	1.9 (0.1) Aa	8.8 (0.3) Ba	4.0 (0.2) Ca
LDT	2.4 (0.2) Aa	8.2 (0.3) Ba	4.3 (0.2) Ca
HDT	3.1 (0.1) Ab	9.9 (0.2) Bb	4.7 (0.2) Cb
MBC (mg g^{-1}C)			
Initial	10.3 (1.09)	12.0 (0.22)	4.1 (0.13)
ZDT	8.9 (0.2) Aa	10.4 (0.8) Ba	3.3 (0.1) Ca
LDT	8.5 (0.3) Ab	7.6 (0.5) Ab	3.6 (0.1) Bb
HDT	8.5 (0.6) Ab	7.6 (0.5) Ab	3.5 (0.3) Bb

[a] Significant differences within each pool, by multiple comparison (Fisher's LSD test, $P < 0.05$), among layers (within treatment row) are indicated by capital letters and among treatments (within layer column) are indicated by lower case letters.

was determined on a Shimadzu total organic carbon (TOC) analyzer. Total inorganic N (NH_4^+-N + NO_3^--N) was measured on an Alpkem nitrogen autoanalyzer. Extractable C and N are expressed in mg g^{-1} of total soil C or N as appropriate. Net N mineralization was calculated as the difference in inorganic N in mg between incubated and initial values expressed g^{-1} of N after incubation.

Microbial biomass was determined by chloroform fumigation direct extraction (Brookes et al., 1985; Vance et al., 1987). Microbial biomass C (MBC) was measured by TOC and microbial biomass N (MBN) was determined following persulfate digestion (Cabrera and Beare, 1993). Microbial biomass results are reported as 0.5 K_2SO_4-extractable C or N of fumigated minus non-fumigated soil expressed in mg g^{-1} of total C or total N, as appropriate.

Microbial activity was determined by substrate induced respiration (SIR) on only A and E horizons using streptomycin and cycloheximide as inhibitors, respectively, of prokaryotic and eukaryotic activity (Anderson and Domsch, 1973). Basal respiration was optimized at 30% gravimetric weight, equivalent to 65–70% of the water holding capacity of the soil layers. Glucose and inhibitor concentrations were optimized by preliminary studies on conditioned field soil. Glucose, streptomycin and cycloheximide, were applied at concentrations of 1, 8, and 10 mg g^{-1} soil dry wt, respectively. Inhibitors were initially applied dry 16 h prior to glucose addition (Johnson et al., 1996). Glucose was applied in solution, increasing soil moisture to 30% gravimetric weight. Minimal supplementary moisture was added to the A horizon soil to aid distribution of inhibitors. Respiration was determined simultaneously on basal, glucose and glucose-plus-inhibitor treatments, after 3 h incubation. Respiration was measured as CO_2 on an infrared gas analyzer (IRGA) using a continuous flow apparatus modified from Cheng and Coleman (1989); Cheng and Virginia (1993). Respiration as CO_2-C in $\mu g\ g^{-1}$ soil dry wt h^{-1} was calculated from averaged flow rate and IRGA reading measured over two minutes. Bacterial respiration was calculated as respiration induced by glucose addition alone (GIR) minus respiration that resulted with addition of glucose plus streptomycin. Fungal respiration was calculated as GIR minus respiration that resulted with addition of glucose plus cycloheximide.

Data were analyzed with Sigma Stat software (Jandel Scientific). Main effects of earthworm density treatment and soil layer were analyzed by two-way ANOVA. Non-normal data were log or square root transformed for analysis. Fisher's LSD test was used as a post-hoc test to analyze multiple comparisons among treatments and layers. Two-way comparisons with a significant interaction occurring between treatment and layer were analyzed within treatment or layer with Fisher's LSD test. Significance of all tests was set at $P < 0.05$.

3. Results

During the incubation period earthworms moved soil from the A horizon layer into the E horizon layer. Burrowing activity maintained open macropores in the A layer and casting activity filled macropores in the E layer. The appearance of the HDT exhibited more earthworm activity than LDT. The ZDT appeared unchanged over the time of the incubation. Minimal activity of earthworms was noted in the B_h layer of both LDT and HDT. Casting material recognized in the soil and collected from earthworms after incubation indicated preferential ingestion of A layer material. All earthworms in the LDT survived, however, one replicate of HDT experienced the loss of three worms. Initial earthworm weight was 2.16 ± 0.20 g per individual. LDT averaged a gain of 0.08 g and HDT averaged a loss of 0.16 g per worm. Outlying values due to earthworm mortality were determined by analysis of residuals and dropped from statistical tests.

Total soil N concentration was lowest in the E horizon initially and, after incubation, increased with increasing earthworm density, up to two-fold in the HDT (Table 1). Final total N was significantly different among all layers, however, was significantly different among all treatments within the E layer only ($df = 4$; $F = 34.9$; $P < 0.0001$; LSD). An increase in total N in the E occurred with an increase in inorganic N and decrease in MBN. Inorganic N was significantly greatest in the E ($F = 20.797$ (log) $P < 0.0001$) and increased with earthworm density ($F = 74.135$ (log), $P < 0.0001$, Table 1). Significant effects on MBN were due to an interaction between treatments and layers ($df = 4$, $F = 2.9$; $P = 0.0407$). Mean MBN was highest in the A layer in the ZDT and lowest in the E layer in the HDT; MBN was significantly reduced in the A layer by LDT and in the E layer by both LDT and HDT (LSD, $P < 0.001$; Table 1). A shift in N balance from the MBN pool to the inorganic pool was apparent in the A layer where there was no change in total N. Net mineralization of N increased significantly with earthworm density in all layers ($F = 116.834$ (log), $P < 0.001$) and had lowest mean value across treatments in the B_h layer ($F = 6.369$ (log), $P = 0.005$; Table 1). Although, net mineralization occurred in all soil layers and treatments, N was immobilized in the ZDT B_h layer only.

Total soil C was lowest initially and remained significantly reduced across treatments in the E layer ($F = 1037.11$; $P < 0.0001$; Table 2). Although treatments had no significant effect across layers, total C in the E layer increased nearly two-fold from the ZDT to HDT. A significant one-way effect of earthworm density on C content in the E layer was obscured by the greater content and variance of total C in the A and B_h layers ($F = 60.1$, $P < 0.0001$). Both C_{sol} and MBC were highest initially in the E layer (Table 2). After incubation C_{sol} was significantly different among the layers but remained greatest in the E ($F = 358.00$, $P < 0.0001$). Across layers mean C_{sol} was significantly greater in HDT than in LDT or ZDT (5.9, 5.0, and 4.9 mg g^{-1} C, respectively; $F = 10.22$; $P = 0.0005$, LSD). After incubation, MBC was less than initial quantities in all layers (Table 1). Final MBC was significantly lower in the B_h layer and was significantly different in the ZDT from LDT and HDT across all layers ($df = 4$, $F = 4.22$, $P = 0.0088$, LSD). MBC in ZDT was highest in A and E layers, but lowest in the B_h layer (Table 1). Earthworm density treatments had no apparent effect on total C in any layer, however, in A and E layers, C balance was shifted from MBC to C_{sol} pools with increasing earthworm density.

As with microbial biomass, basal soil respiration was significantly greater in A than in E layer soil (0.82 ± 0.02 and 0.24 ± 0.04 μg CO_2-C g^{-1} h^{-1}, respectively; $F = 139.40$, $P < 0.001$). There were no significant differences in GIR between layers or treatments, however respiration was stimulated by 228–237% in the A layer and by 80–137% in the E layer. Respiration was significantly greater in the A layer for both bacteria ($F = 326.497$, $P < 0.001$) and fungi ($F = 512.577$, $P < 0.001$). Stimulation of both bacterial and fungal respiration by LDT and HDT compared to ZDT was most pronounced in the E layer (Fig. 1). Bacterial respiration was significantly stimulated in HDT and LDT over ZDT on average by 30% in the A layer and by 450% in the E layer ($F = 8.714$, $P = 0.002$). Fungal respiration in LDT and HDT was significantly stimulated over ZDT on average by 13% in the A layer and by 123% in the E layer ($F = 5.814$ (sqrt), $P < 0.012$). Fungal respiration (1.50 ± 0.06 μg CO_2-C g^{-1} h^{-1}) was significantly greater than bacterial (1.32 ± 0.06 μg CO_2-C g^{-1} h^{-1}) across treatments in the A layer ($F = 7.34$, $P = 0.014$). The B:F ratio ranged from 0.80 to 0.93 in the A layer, from 0.80 to 0.99 in the E; B:F was higher in LDT and HDT, but the effects were not significant.

4. Discussion

The major effect of *D. mississippiensis* activity was the obvious physical disruption of the reconstructed soil layers. Cast material in the E layer corresponded with removal of A layer material. Evidently, the near two fold increases in total

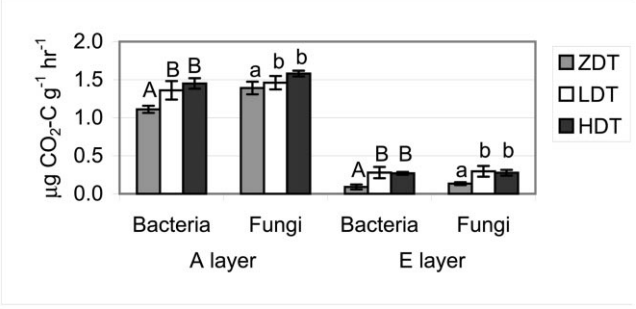

Fig. 1. Bacterial and fungal respiration of A and E layer soil by earthworm density treatment (ZDT = zero, LDT = three earthworms, and HDT = six earthworms). Bars show one standard error. See text for calculation of respiration measurements. Different letters within a component of respiration indicate significant differences among earthworm density treatment means across layers (Fisher's LSD) at $P < 0.05$. For HDT means in the A layer $n = 3$, for all other means $n = 4$.

C and N in the E layer resulted from casting activity. In comparable soils, total C and N in casts of *D. longa* were significantly greater than in bulk soil (Hendrix and Callaham, 1994). The redistribution of A layer material by earthworms provided a source of soil organic matter and microbial biomass for the E layer. Total C and N pools within A and B_h layers were unchanged across treatments, however shifts in proportion of extractable and microbial pools in LDT and HDT compared to ZDT indicated C and N were mobilized in all soil layers. Nutrient mobilization in the E layer was clearly due to both organic matter enrichment and effects associated with passage of this material through the earthworm gut. In the E layer, the distinction between NEP and GAP, made through experiments of Devliegher and Verstraete (1995) was not directly tested, however, the function of both processes was recognized in this study.

Earthworm activity had significant effects on N dynamics. *D. mississippiensis* activity contributed to net N mineralization in all soil layers (Table 1). In the E layer, much more of the N deposited in casts was introduced in mineral rather than microbial forms. Midwestern species of *Diplocardia* studied by James (1991) released great amounts of inorganic N through cast production. Based on organic matter enrichment we estimated that *D. mississippiensis* could have released between 76 and 173 μg inorganic N per gram of cast. These values are close to those of James (1991) who found that around 40–170 μg of total NH_4^+-N and NO_3^--N could be added to the soil per gram of cast. In the A layer, an increase in inorganic N (mineralized N) in the LDT, with a significant decrease in MBN and no change in total N indicated N was mobilized in part from microbial to mineral pools. Decreased MBN associated with increased total N in the E layer could be attributed to organic matter enrichment from the A horizon. Decreased microbial N was typical in other earthworm incubations (Bohlen and Edwards, 1995; Willems et al., 1996; Hendrix et al., 1998). Decreased microbial N was attributed to residue incorporation and increased turnover of organic matter N through the microbial biomass with earthworm activity (Hendrix et al., 1998). However, Brown et al. (1995) found that *Lumbricus rubellus* contributed to N mobilization from residue to soil but with no effect on microbial or mineral N forms.

Mobilization of C detected as changes in C_{sol} and MBC pools occurred with earthworm activity. Decreased MBC indicated less immobilization of C within the microbial biomass with earthworm activity. Stimulation of bacterial and fungal respiration with earthworm activity suggested that mobilization of C was associated with microbial turnover, however, net C mineralization, as a fractional change in total C, was immeasurable. Soil respiration was substantially increased in the presence of earthworms in a 16 day incubation in accordance with increased microorganism (protozoan) density (Binet et al., 1998). However, increased microbial activity was not associated with microbial biomass changes in a 225 day incubation with *Octolasian lacteum* (Scheu, 1993). Bohlen and Edwards (1995) found that organic matter amendments of green and manure fertilizers to plots with high densities of earthworms produced more CO_2 respiration than plots without amendments. As with N mineralization, nutrient enrichment may also have lead to increased C turnover in the E layer.

Change in microbial biomass C and N supported a possible change in the microbial composition of A and E horizons. Decreased MBC and MBN possibly resulted from greater bacterial activity. Saetre (1998) found decreased microbial biomass and associated N with a decrease in soil fungi and increase in actinomycetes. Casting activity may have introduced more bacteria, which would result from stimulation of microbial growth and activity inside the earthworm gut (Parle, 1963a,b; Barois and Lavelle, 1986; Barois, 1992; Kristufek et al., 1992; Fischer et al., 1995). However, both bacterial and fungal respiration were stimulated in treatments with earthworms. Fungal respiration was greater than bacterial respiration reflecting the fungal dominance of microbial biomass typical in pine habitats. Calcium, a proposed limiting nutrient in the ANF system, is bound by fungal oxalates (Cromack et al., 1979; Fox and Comerford, 1992; Delucia et al., 1997). To obtain required calcium, *Diplocardia* spp. may be capable of ingesting fungal biomass, however, there was no evidence that fungal activity was affected by direct feeding of earthworms. The SIR technique (Anderson and Domsch, 1973) was used previously to show increased B:F ratios with activity of *O. lacteum* and *Dendrobaena octaedra* (Lumbricidae) under laboratory conditions (Scheu and Parkinson, 1994). Although our results indicated a shift towards bacteria, as B:F ratio increased with earthworm density, SIR was inadequate for measuring statistically significant differences. As a residual of gut passage, nutrient enrichment and increased microorganism biomass or activity, including viable propagules of fungi, were found previously in earthworm castings, including those produced by *Diplocardia* spp. (Parle, 1963a; Shaw and Pawluk, 1986; James, 1991; Reddell and Spain, 1991; Daniel and Anderson, 1992; Tiwari and Mishra, 1993; Hendrix and Callaham, 1994). Although, bacterial respiration was more greatly stimulated than fungal respiration by earthworm activity in A and E layers, there was no indication of strong selective changes due to GAP. Stimulation and maintenance of greater fungal respiration in earthworm treatments could have been caused by fungal succession in aging cast material (Parle, 1963a; Tiwari and Mishra, 1993; Tiunov and Scheu, 2000a,b).

We proposed that *D. mississippiensis*, like other well-studied species, would stimulate microbial biomass and activity and exhibit selective effects on microbial components. This paper showed that earthworm activity, primarily casting of A layer material, increased C and N content of the E layer over two fold. Reduced nutrient immobilization by the microbial biomass was indicated by reduced biomass in proportion to total C and N pools with earthworm activity. A

strong relationship between *D. mississippiensis* and N turnover was apparent with shifts in balance of mineral and microbial N pools. Increased availability of mineralized N with earthworm activity, established by this study, conceivably induced the increased native plant biomass and N content previously observed with activity of *D. mississippiensis* (Callaham and Hendrix, 1998). This study established that *D. mississippiensis* functions comparably to lumbricid species, in contributing to N availability (Robinson et al., 1992; Scheu, 1993; Willems et al., 1996). The effects of *D. mississippiensis* were likely due to the mixing of A horizon soil, a source of greater microbial biomass and soil organic matter, into the E horizon soil. SIR with inhibitors indicated that earthworm activity caused significant stimulation of microbial respiration in the A and E layers. Similar to experiments of Fischer et al. (1995); Wolter and Scheu (1999) there was no evidence that earthworms fed on or selectively stimulated any specific microbial component. This study established an important role for *D. mississippiensis* in nutrient availability in its native soil. Although average field densities of *D. mississippiensis* are relatively low (ca. 3 m^{-2}), their highly clumped distributions suggest potential for significant localized effects on nutrient dynamics, for example, in plant rhizospheres. Reduced densities of *D. mississippiensis* caused by harvesting (Hendrix et al. 1994) may impact forest regeneration in burn management areas.

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